Format to CLSI Standards
GP2-A5 (Formerly NCCLS)
Vol. 26 No. 12
Issue Date:10/15/09

QUANTA Plex™ ENA Profile 5 708920

For *In Vitro* Diagnostic Use
CLIA Complexity: High

Principles of the Procedure

Native Sm, RNP and Scl-70 from calf thymus and recombinant human SS-B are each bound to different, fluorescently "colored" beads. Because it has been reported that autoantibodies to both SS-A 52 and SS-A 60 antigens are diagnostically important[6], native SS-A 60 from calf thymus and recombinant SS-A 52 are used on separate colored beads. The six different antigen coated beads are mixed together and put into wells of a microwell plate under conditions that preserve the antigens in their reactive state. Pre-diluted controls and diluted patient sera are added to separate microwells, allowing any Sm, RNP, SS-A 52, SS-A 60, SS-B and Scl-70 autoantibodies present to bind to the immobilized antigen. Then an anti-human IgG conjugated to a fluorescent probe is added to each microwell. A second incubation allows the anti-human IgG fluorescent conjugate to bind to any patient autoantibodies that have become attached to the antigen on the beads. The samples are then measured in the Luminex™ flow analyzer. This flow analyzer can discriminate the color of each bead from the others as well as measure the fluorescent intensity of the conjugate on each bead.[7] The conjugate's fluorescent intensity is proportional to the amount of labeled anti-human IgG bound to the patient autoantibodies on the bead. Each antigen can be semi-quantitated by comparing the fluorescent intensity of the patient sample with the fluorescence of the corresponding Calibrator. An anti-IgG-coated control bead is included in each microwell to ensure that false negative results due to operational errors are detected.

Antinuclear autoantibodies (ANA) are found in a wide variety of connective tissue diseases. Testing for ANA on HEp-2 cells or an ANA ELISA serves as a sensitive screening assay.[1] While ANA testing is an excellent screening test for systemic lupus erythematosus (SLE), a negative result virtually rules out active SLE[2], it is by no means a specific test. Follow-up testing is typically done on sera that yield positive ANA results. Six of the more common autoantibodies react specifically with Sm, RNP, SS-A 52, SS-A 60, SS-B and Scl-70 extractable nuclear antigens (ENAs). Autoantibodies to these ENAs can contribute significant diagnostic and prognostic information when evaluating patients suspected of a variety of connective tissue diseases such as SLE[2], Sjögren's syndrome[3], Scleroderma[4], Mixed Connective Tissue Disease[5] and Polymyositis.[6]
A variety of methods including ELISA, Ouchterlony double diffusion, Western blot and passive agglutination have been used to detect antibodies to Sm, RNP, SS-A 52, SS-A 60 SS-B and Scl-70. The fluorescent immunoassay (FIA) technique employed by the QUANTA Plex™ ENA Profile 5 is objective, semi-quantitative, and can be conveniently used to simultaneously test large numbers of patients on each of these 6 ENAs. Semi-quantitative results are obtained for each autoantibody reactivity.

The QUANTA Plex™ ENA Profile 5 is a fluorescent immunoassay for the semi-quantitative detection of Sm, RNP, SS-A, SS-B and Scl-70 autoantibodies in human serum. The presence of these antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of systemic lupus erythematosus and the related connective tissue diseases Sjögren's Syndrome and Scleroderma.

Reagents

1. Polystyrene microwell plate, 12 (1 x 8) microwell strips with holder, containing beads of 7 different colors. Each of the colored beads is coated with a different purified antigen (Sm, RNP, SS-A 52, SS-A 60, SS-B, Scl-70 and an anti-IgG control), in a foil package containing desiccants
2. QUANTA Plex™ Negative Control, 1 vial of buffer containing preservative and human serum with no human IgG antibodies to antigens in the ENA Profile 5, prediluted, 1.2mL
3. ENA Profile 5 Calibrator, 1 vial of buffer containing preservative and human serum antibodies to Sm, RNP, SS-A 52, SS-A 60, SS-B and Scl-70 antigens, prediluted, 1.2mL

INOVA Diagnostics, Inc.
9900 Old Grove Road
San Diego, CA 92131-1638, USA
Tel. +1 858 586 9900 – Fax: +1 858 586 9911
http://www.inovadx.com

Reagents (continued)

4. ENA Profile 5 Positive, 1 vial of buffer containing preservative and human serum antibodies to Sm, RNP, SS-A 52, SS-A 60, SS-B and Scl-70 antigens, prediluted, 1.2mL
5. HRP Sample Diluent, 1 vial – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives, 50mL
6. Fluorescently labeled IgG Conjugate (goat), anti-human IgG (fc specific), 1 amber vial – lyophilized powder, containing buffer, protein stabilizers and preservatives. Refer to the Methods section for reconstitution instructions.
7. QUANTA Plex™ Conjugate Diluent, 1 vial - colored pink, containing buffer, protein stabilizers and preservatives, 7mL

Materials provided

1 ENA Profile 5 microwell plate, 12 (1 x 8) microwell strips, with holder
1 1.2mL prediluted, ready to use QUANTA Plex™ Negative Control
1 1.2mL prediluted, ready to use ENA Profile 5 Calibrator
1 1.2mL prediluted, ready to use ENA Profile 5 Positive
1 50mL HRP Sample Diluent
1 Bottle of lyophilized Fluorescent Conjugate (goat), anti-human IgG
1 7mL QUANTA Plex™ Conjugate Diluent

Additional Materials Required But Not Provided

Sheath Fluid for Luminex™ flow analyzer
Micropipet to deliver 5 and 500µL
Disposable micropipet tips
Test tubes for patient sample dilutions, 1 to 4mL volume
Distilled or deionized water
Luminex™ flow analyzer
8-channel Electronic pipet to deliver 5, 30, 45, 50 and 60µL or automated pipetter/diluter

Specimen

Specimen Collection

This procedure should be performed with a serum specimen. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Grossly hemolyzed or lipemic serum should be avoided.
Following collection, the serum should be separated from the clot. CLSI (formerly NCCLS) Document H18-A3 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2 - 8°C. 3). If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Special Safety Precautions/Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused microwell strips with antigen-coated beads should be securely resealed in the foil pouch containing desiccants and stored at 2-8°C.

Procedural Notes:

Warnings

1. WARNING: The HRP Sample Diluent and controls contain a chemical (0.02% chloramphenicol) known to the State of California to cause cancer.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Negative Control and the ENA Profile 5 Calibrator and Positive should be handled in the same manner as potentially infectious material.[8]
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. Use appropriate personal protective equipment while working with the reagents provided.
5. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this kit may lead to inconsistent results. All controls are kit lot number specific.
3. Adaptation of this assay for use with certain automated liquid sample processors was shown to yield equivalent results to those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the reproducibility of the mixing technique, the Luminex™ flow analyzer used to measure the results and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
5. Strict adherence to the protocol is recommended.
6. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
7. Unacceptably low fluorescence may be observed following multiple uses from a single bottle of fluorescent conjugate over a period of time. It is important to follow all recommended fluorescent conjugate handling procedures to prevent this occurrence.
8. Chemical contamination of the fluorescent conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent will cause degradation of the fluorescent conjugate over time. Thoroughly rinse all equipment or instruments with distilled or deionized water after the use of chemical cleaners/disinfectants.

Quality Control

1. The ENA Profile 5 Calibrator and Positive Control and the QUANTA Plex™ Negative Control, should be run with every batch of samples to ensure that all reagents and procedures have performed properly.
2. Note that since the ENA Profile 5 Calibrator and Positive Control and QUANTA Plex™ Negative Control are prediluted, they do not control for procedural methods associated with specimen dilutions.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing them at $\leq$ -20° C.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated. The value in Luminex Units (LU) of the Calibrator for each antigen is found on the box label for that kit. Refer to the formula in the Calculation of Results section to determine the LUs of the QUANTA Plex™ Negative Control and the Positive Control.
   a. The value of the QUANTA Plex™ Negative Control must be < 20 LU on each of the 6 antigens, and between 10 and 50 LU on the anti-IgG bead.
   b. The value of the ENA Profile 5 High Positive must be $\geq$ 100 LU and ≤ 300 LU on each of the 6 antigens, and between 10 and 50 LU on the anti-IgG bead.
   c. The anti-IgG control bead is meant to ensure that false negative patient results due to operational errors are detected. The possibility exists that patient sample and/or conjugate was not added to the patient sample well if the patient sample's anti-IgG bead is less than 3 LU. In this case the patient should be re-tested to confirm the negative result.
5. The QUANTA Plex™ Negative Control and the ENA Profile 5 Calibrator and Positive Control are intended to monitor for substantial reagent failure. The user should refer to CLSI (formerly NCCLS) Document C24-A3 for additional guidance on appropriate QC practices.
6. If desired, a well with HRP Sample Diluent but no serum can be run to confirm that the anti-IgG control bead will detect a well with no serum. This serum free control should be < 3.0 LU on the anti-IgG control bead and negative on all other beads.

Using the Luminex™ Flow Analyzer

1. See the user's manual provided with the Luminex™ for detailed instructions on running the Luminex™ flow analyzer and the Luminex™ Integrated System (IS) Version or the Version 2.0 or higher software program. The data for the ENA Profile 5 were collected using IS Version 1.7 and 2.2.329. For additional information and for troubleshooting problems with this assay, contact INOVA Diagnostics, Inc. technical service at the address or telephone number found on the last page of the Direction Insert. Brief Luminex™ flow analyzer operating instructions are provided below.
2. Calibrate the Luminex™ using the Calibration and Control beads supplied by Luminex Corporation at least once per month and verify that calibration was successful. In addition, calibrate the Luminex™ if the delta calibration temperature is more than 3 degrees, if the assay controls are out of range or as needed.

3. The Luminex™ takes 30 minutes to warm-up after being turned on. When the warm-up period is completed, perform the prime, alcohol flush and wash operations recommended by the manufacturer.
4. If using IS Version 2.0 software or higher, load the "ENA Profile 5" template and ensure that all lot information is correct. If necessary, update the lot information. If using Version 1.7 software, set the parameters as follows. The bead colors are SS-A 52 = 19, SS-A 60 = 17, SS-B = 7, Sm = 8, RNP = 9, Scl-70 = 11, anti-IgG = 42. Set the events per bead to 50, the sample size to 50µL, the flow rate to 60µL/minute (fast), and the gate at approximately 7500 to 17000. The median values are used for the Median Fluorescent Intensity (MFI).
5. Input the sample names either manually or by clicking on "Load Pa List".
6. Load the plate into the XY platform of the Luminex™.
7. Run the Luminex™ by clicking the "Start Plate".
8. When finished for the day, perform the sanitize and soak operations prior to turning the instrument off.

Method: Before you start

1. For programming information for automated equipment, contact INOVA Diagnostics Technical Services.
2. Turn on the Luminex™ flow analyzer, ensure that it is warmed-up, and perform all daily maintenance operations as previously described. If necessary, calibrate the instrument and verify that the calibration was successful.
3. Bring all reagents and patient samples to room temperature (20-26^{o}C) and mix them well.
4. If the anti-human IgG fluorescent conjugate has not been reconstituted, add 6mL of QUANTA Plex Conjugate Diluent to the amber vial containing the lyophilized powder and swirl the container for approximately 30 seconds to dissolve the contents. The reconstituted fluorescent conjugate is stable for 3 months at 2-8°C. **Do not freeze.**
5. Make sure that the sheath fluid container in the Luminex™ is filled with Sheath Fluid available from Luminex Corporation.
6. Prepare a 1:101 dilution of each patient sample by adding 5µL of each serum to 500µL of HRP Sample Diluent, then vortex to thoroughly mix the solution. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the QUANTA Plex™ Negative Control and ENA Profile 5 Low and High Positives.
7. Determination of the presence or absence of Sm, RNP, SS-A 52, SS-A 60, SS-B and Scl-70 antibodies using arbitrary units requires one microwell each for the Negative and Positive Controls, two microwells for the Calibrator and one microwell for each patient sample. It is recommended that samples be run in singleton.

Assay Procedure

1. **All reagents must be brought to room temperature (20-26°C) prior to beginning the assay.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor and light.**
2. Add 45µL of HRP Sample Diluent to each microwell that will contain a patient sample. **Do not** add HRP Sample Diluent to the first four microwells, as these will contain the QUANTA Plex™ Negative Control, the ENA Profile 5 Calibrator (in duplicate), and the ENA Profile 5 Positive Control. **Do not** add the diluted patient samples to the microwells at this time.
3. Maintain one of the following timing sequence when adding controls, samples, and conjugate to the microwells. Because the samples are read sequentially at a rate of approximately 1 sample every 19 seconds (or one 8-well strip in approximately 2½ minutes) by the Luminex™, the patient samples and also the conjugate must be added to the microwells at this rate to minimize any front to back assay variation. If the controls, samples or the conjugate are added one at a time, stagger each addition of these to the next microwell by 19 seconds. If controls, samples or the conjugate are added 8 at a time to a strip, stagger each addition of any of these to the next strip by 2 ½ minutes. Both of these timing schemes will take approximately 30 minutes for the addition of the samples to all 12 strips of an entire plate and will minimize front to back assay variation.
4. Vortex, then add 50µL of each of the following: The **pre-diluted** QUANTA Plex™ Negative Control to the first microwell, the ENA Profile 5 Calibrator to the second and third microwells, and the ENA Profile 5 Positive Control to the fourth microwell. An electronic pipet must be used when manually adding samples and mixing the beads. Use one of the timing sequences for adding the controls as described in step 3. **Vigorously pipet at least 30µL of the QUANTA Plex™ Negative Control and ENA Profile 5 Calibrator and Positive Control up and down four times in order to mix the beads and the controls in each microwell.** The 30-minute incubation time begins after adding the QUANTA Plex™ Negative Control into the **first** microwell.

5. Immediately continue the assay by adding 5μL of diluted patient serum to the appropriate microwells (note: this makes a 1:1010 final dilution of the patient serum). Maintain the same timing sequence as used in step 4. **Mix the diluted patient sample and the HRP Sample Diluent in the microwell by vigorously pipetting at least 30μL of the contents of the microwell up and down four times.** Continue timing the incubation for 30 minutes from the time of the addition of the QUANTA Plex™ Negative Control. Place the microwell strips at room temperature, on a level surface, and away from direct sunlight for the remainder of the incubation period.
6. At the end of the first incubation period, add 50μL of the fluorescent Conjugate to each microwell and **vigorously pipet at least 60μL of the contents of the microwell up and down four times.** Maintain the same timing sequence for adding the conjugate that was used in steps 4 and 5. The conjugate should be removed from the bottle using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the microwell strips for 30 minutes at room temperature, on a level surface and away from direct sunlight. The incubation time begins after the first conjugate addition.
7. Within one hour after completion of the 30-minute fluorescent conjugate incubation, read the ENA Profile 5 plate on the Luminex™ as detailed in the section above, "Using the Luminex™ Flow Analyzer."

Reporting Results:

Interpretation of Results

The FIA is very sensitive to technique and is capable of detecting small differences in patient populations. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Plex™ ENA Profile 5. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG autoantibody levels cannot always be correlated to an endpoint titer."

Calculations

Calculation of Results

The MFIs for all controls and patient samples for each antigen are first determined. The reactivity in LU of a given sample for each type of antigen can then be calculated by the following formula. Divide the MFI of the sample by the MFI of the ENA Profile 5 Calibrator for that antigen and multiply the result by the number of LU assigned to the ENA Profile 5 Calibrator for that antigen. The LU of the Calibrator for each antigen is found on the box label for that kit lot. The following example is for determining anti-Sm reactivity. Use the equivalent formula for each of the other antigens.

$$\text{Sample Value (in LU)} = \frac{\text{Sample MFI for Sm}}{\text{Sm Calibrator MFI}} \times \text{Sm Calibrator value (in LU)}$$

The sample can then be classified according to the table below.

	LU
Negative	<20
Weak Positive	20-49
Moderate Positive	50-100
Strong Positive	>100

Reactivity is related to the quantity of autoantibody present on the bead in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in fluorescent reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). In addition, the amount of total IgG in a patient's serum affects the measured MFI. If a more accurate quantitation of patient autoantibody is required, the sample should be run on a quantitative test.

Expected Values

The ability of the QUANTA Plex™ ENA Profile 5 test to detect Sm, RNP, SS-A 52, SS-A 60, SS-B and Scl-70 antibodies was evaluated by comparison to commercially available ELISA tests from INOVA Diagnostics, Inc. Results of the ELISAs and each of the QUANTA Plex™ ENA Profile 5 tests were determined to be positive if the patient sample was greater than or equal to 20 LU and negative if less than 20 LU.

Normal Range

One hundred sixty samples from normal blood donors were run on the QUANTA Plex™ ENA Profile 5 test. One hundred fifty eight normal samples were negative on the SS-A 52 portion of the QUANTA Plex™ test. The highest sample had a value of 49 LU. The average value was 3.8 LU with a standard deviation (SD) of 4.9. The cutoff is 3.3 SD above the average.

One hundred fifty nine of the normal samples were negative on the SS-A 60 portion of the QUANTA Plex™ test. The highest sample had a value of 31 LU. The average value was 2.5 LU with a SD of 2.8. The cutoff is 6.3 SD above the average.

One hundred fifty nine of the normal samples were negative on the SS-B portion of the QUANTA Plex™ test. The highest sample had a value of 82 LU. The average value was 3.4 LU. The SD was 6.8. The cutoff is 2.4 SD above the average.

All one hundred sixty of the normal samples were negative on the Sm portion of the QUANTA Plex™ test. The average value was 2.6 LU with a SD of 2.4. The cutoff is 7.2 SD above the average.

All one hundred sixty of the normal samples were negative on the RNP portion of the QUANTA Plex™ test. The average value was 2.3 LU with a SD of 2.4. The cutoff is 7.4 SD above the average.

One hundred fifty seven of the normal samples were negative on the Scl-70 portion of the QUANTA Plex™ test. The highest sample had a value of 48 LU. The average value was 5.5 LU with a SD of 4.7. The cutoff is 3.1 SD above the average.

Comparison between QUANTA Plex™ and ELISA Assays

To determine the positive and negative agreement of the assays in clinically defined patient samples, 288 patients with either SLE, Sjögren's syndrome or scleroderma were tested by the QUANTA Plex™ ENA Profile 5 technique and the 5 corresponding ELISAs.

SLE, SS and Scl n=288	INOVA ENA Profile 5 (Sm)		
Sm ELISA 96.2% the same		Negative	Positive
	Negative	244	4*
	Positive	7**	33

*2 were low positive and 2 were a moderate positive.
**6 were low positive and 1 was a moderate positive.

SLE, SS and Scl n=284^	INOVA ENA Profile 5 (RNP)		
RNP ELISA 94.0% the same		Negative	Positive
	Negative	203	0
	Positive	17*	64

^4 samples were not tested because of insufficient volume.
*11 were low positive, 2 were moderate and 4 were high.

SLE, SS and Scl n=288	INOVA ENA Profile 5 (SS-A 52 and SS-A 60)		
SS-A ELISA 97.6% the same		Negative	Positive
	Negative	205	2*
	Positive	5**	76

*Both were low positive. ** 1 was low positive, 4 were moderate

SLE, SS and Scl n=288	INOVA ENA Profile 5 (SS-B)		
SS-B ELISA 97.9% the same		Negative	Positive
	Negative	255	1*
	Positive	5**	76

*This was low positive. **4 were low positive and 1 was moderate.

SLE, SS and Scl n=288	INOVA ENA Profile 5 (Scl-70)		
Scl-70 ELISA 96.2% the same		Negative	Positive
	Negative	241	1*
	Positive	10**	36

*This was low positive. ** 7 were low positive, 2 were moderate and 1 was high.

Positive, Negative and Total Percent Agreement
All the data above plus data from patients with rheumatoid arthritis were used to calculate the relative sensitivity of each QUANTA Plex™ test to its corresponding ELISA.

All Samples N=460	Both Neg	Both Pos	ELISA Neg Q.Plex Pos	ELISA Pos Q.Plex Neg	Relative Sensitivity	Relative Specificity	Percent Agreement
Sm	416	33	4	7	83%	99%	97.6%
RNP*	374	64	0	18	78%	100%	96.1%
SS-A	375	76	4	5	94%	99%	98.0%
SS-B	426	27	2	5	84%	99%	98.5%
Scl-70	409	38	2	11	78%	99%	97.2%

*N = 456 for RNP, 4 samples were not tested on ELISA due to insufficient volume.

Precision and Reproducibility

Inter-assay and Intra-assay variation. For inter-assay variation, a number of samples were assayed in 6 tests run on different days. The variation in two different samples for each test specificity is listed in the table below. For intra-assay variation, a number of samples were assayed 8 times each on a single assay. The variation in two different samples for each test specificity is listed in the table below.

Inter-assay variation			Intra-assay variation		
Antigen	Average LU	% C.V.	Antigen	Average LU	% C.V.
SS-A 52	32	17%	SS-A 52	33	14%
SS-A 52	173	19%	SS-A 52	163	4%
SS-A 60	26	8%	SS-A 60	26	13%
SS-A 60	203	5%	SS-A 60	202	2%
SS-B	27	12%	SS-B	28	11%
SS-B	266	10%	SS-B	275	1%
Sm	29	8%	Sm	28	8%
Sm	209	12%	Sm	216	6%
RNP	39	4%	RNP	40	8%
RNP	144	9%	RNP	156	4%
Scl-70	26	11%	Scl-70	26	11%
Scl-70	202	9%	Scl-70	199	5%

Limitations of the Procedure

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Not all SLE, Sjögren's syndrome or scleroderma patients are positive for Sm, RNP, SS-A 52, SS-A 60, SS-B or Scl-70 antibodies.
3. Results of this assay should be used in conjunction with clinical findings and other serological tests.
4. Failure to adequately mix the controls and/or the diluted serum samples with the preserved beads in the plate may yield higher %C.V. values than those typically found in ELISA assays.
5. After the half-hour incubation with the fluorescent conjugate, there is approximately a 10% further increase in fluorescence for every additional half-hour of incubation time.
6. The performance characteristics of this assay have not been established for matrices other than serum.
7. Failure to maintain consistent reagent addition timing may result in increased front-to-back assay variation.

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INOVA Diagnostics, Inc.
9900 Old Grove Road
San Diego, CA 92131

858-586-9900
628920 Rev. 8
2009/APR